2012

41st National Poultry Improvement Plan Conference

Proposed Changes Booklet

NATIONAL POULTRY IMPROVEMENT PLAN

September 25-27, 2012
New Orleans, LA

Present provisions of the National Poultry Improvement Plan are contained in the U.S. Department of Agriculture publication, "National Poultry Improvement Plan and Auxiliary Provisions," and in Title 9 CFR parts 145, 146, 147 and 56.

The detailed procedure for making changes in the Plan is described in the auxiliary provisions, sections 147.41 through 147.48. Copies of the "National Poultry Improvement Plan and Auxiliary Provisions" are available from each Official State Agency or from the National Poultry Improvement Plan staff, Animal and Plant Health Inspection Service, Veterinary Services, Suite 300, 1506 Klondike Road, Conyers, Georgia 30094 or at the NPIP web site http://www.aphis.usda.gov/animal_health/animal_dis_spec/poultry/

Proposed changes and supporting statements in this publication were submitted as provided in section 147.44. They are compiled in this publication for consideration at the 2012 National Plan Conference. This publication is distributed well in advance of the conference so that participants and other interested persons may review the proposed changes and inform conference delegates of their wishes regarding the proposals.

Some proposed changes have a line drawn through a portion of the words while other portions are underscored. The line through the words indicates that they are part of the present provision but would be deleted if the proposal were adopted. The underscored words are the proposed additions to that provision.

Each State is entitled to one official delegate for each of the subparts, B, C, D, E, F, G, H and I of part 145 and B, C, D and E of part 146. Each delegate will act on proposals affecting the provisions of the program which he represents. For reference purposes, delegates are designated as follows:

subpart B delegates - representing egg-type chickens.
subpart C delegates - representing meat-type chickens.
subpart D delegates - representing turkeys.
subpart E delegates - representing waterfowl, exhibition poultry, and game birds.
subpart F delegates - ostrich, emu, rhea, and cassowary
subpart G delegates- primary egg-type chickens
subpart H delegates- primary meat-type chickens
subpart I delegates- representing meat-type waterfowl
subpart 6B delegates- commercial table-egg layers
subpart 6C delegates- commercial meat-type chickens
subpart 6D delegates- commercial meat-type turkeys
subpart 6E delegates-waterfowl, upland Gamebirds

This compilation of proposed changes includes, in the margin adjacent to the section reference for each proposal, the delegate entitled to vote on the proposal. Some of the changes proposed apply equally to all participants in which case conference action will be determined by the <u>combined</u> vote of all delegates.

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Proposal No. 1

Delegates:
D

§ 145.43(g) ***U.S. H5/H7 Avian Influenza Clean.*** This program is intended to be the basis from which the turkey breeding industry may conduct a program for the prevention and control of the H5/H7 subtypes of avian influenza. It is intended to determine the presence of the H5/H7 subtypes of avian influenza in breeding turkeys through routine surveillance of each participating breeding flock. A flock, and the hatching eggs and poults produced from it, will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a primary breeding flock in which a minimum of 30 birds have been tested negative to the H5/H7 subtypes of avian influenza as provided in §145.14(d) when more than 4 months of age and prior to the onset of egg production. To retain this classification:

(i) A sample of at least 30 birds must be tested negative at intervals of 90 days; or

(ii) A sample of fewer than 30 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 30 birds are tested within each 90-day period.

(2) It is a multiplier breeding flock in which a minimum of 30 birds have been tested negative to the H5/H7 subtypes of avian influenza as provided in §145.14(d) when more than 4 months of age and prior to the onset of egg production. To retain this classification:

(i) A sample of at least 30 birds must be tested negative at intervals of 90 days; or

(ii) A sample of fewer than 30 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 30 birds are tested within each 90-day period.

(3) ~~During each 90 day period, all spent fowl, up to a maximum of 30, mucst be tested and found negative within 21 days prior to movement to slaughter.~~

(3) All spent fowl being marketed for meat that have been tested as required for the AI Clean program for turkey breeders shall be tested at a rate of 6 birds per flock within 21 days prior to slaughter.

(4) For both primary and multiplier breeding flocks, if a killed influenza vaccine against avian influenza subtypes other than H5 and H7 is used, then the hemagglutinin and the neuraminidase subtypes of the vaccine must be reported to the Official State Agency for laboratory and reporting purposes.

Reason: Though the normal requirement for H5/H7 AI Clean certification for turkey breeders allows the birds to be tested once every 90 days at a minimum, most commercial turkey breeders holding this classification are tested much more frequently, some as often as every three weeks. The current rule requires the testing of 30 birds when spent fowl are sent to slaughter. When birds are tested as frequently as is typical for commercial turkey breeders, this amount of testing seems excessive. We are requesting that birds which are tested and qualify as H5/H7 AI Clean for their reproductive life be treated as meat birds for pre-slaughter testing and thus be required to test 6 birds per flock within 21 days of slaughter.

Sponsor: Dr. Eric Gonder
Butterball Turkeys

Proposal No. 2

Delegates

D **145.43*(e) U.S. M. Synoviae Clean.***

(1) All birds, or a sample of at least 100 birds from flocks of more than 100 and each bird in flocks of 100 or less, have been tested for M. synoviae when more than 12 weeks of age in accordance with the procedures in §145.14(b): Provided, That to retain this classification a minimum of 30 samples from male flocks and 60 samples from female flocks shall be retested at 28–30 weeks of age and at 4–6 week intervals thereafter. It is recommended that samples be collected from birds with clinical signs such as lameness, swollen hocks, swollen foot pads, synovitis or exudate in the joints or tendon sheaths.

(2) When reactors to the official test are found and can be identified, tracheal swabs and their corresponding blood samples from 10 (all if fewer than 10) reacting birds shall be submitted to an authorized laboratory for serological and cultural examination. If reactors cannot be identified, at least 30 tracheal swabs and their corresponding blood samples shall be submitted. In a flock with a low reactor rate (less than five reactors) the reactors may be submitted to the laboratory within 10 days for serology, necropsy, and thorough bacteriological examination. When reactors to the official test are found, the procedures outlined in §147.6 of this chapter will be used to determine the status of the flock.

~~(3) Flocks located on premises which, during 3 consecutive years, have contained breeding flocks qualified as U.S. M. Synoviae Clean, as described in paragraph (e)(1) above, may qualify for this classification by a negative blood test of at least 100 birds from flocks of more than 100 and each bird in flocks of 100 or less, when more than 12 weeks of age, and by testing a minimum of 30 samples from male flocks and 60 samples from female flocks at 28–30 weeks of age and at 45 weeks of age.~~

Reason: MS is a difficult disease to diagnosis in breeder turkeys with few if any clinical signs in breeder flocks. An MS Clean surveillance program requires that the proper samples be collected every 4 – 6 weeks. This proposal will help to validate the MS Clean status of turkey breeding flocks.

Proponent: Dr. Dale Lauer
Minnesota Board of Animal Health

Proposal No. 3

Delegates:
Combined **§145.1 and 146.1 Definitions**

Federal Reference Laboratories- the National Veterinary Services Laboratories (NVSL) in Ames, Iowa, including NVSL's Diagnostic Virology Laboratory and Diagnostic Bacteriology Laboratory

Reason: Defines Federal Reference Laboratories referenced in Parts 145 and 146.

Sponsor: Senior Coordinator

Proposal No. 4

Delegates:

Combined

§**145.14 (d)(1)** ***For avian influenza*.** The official tests for avian influenza are described in paragraphs (d)(1) and (d)(2) of this section.
(1) *Antibody detection tests*—(i) *Enzyme-linked immunosorbent assay (ELISA)*. ELISA must be conducted using test kits approved by the Department and the Official State Agency and must be conducted in accordance with the recommendations of the producer or manufacturer. The AGID test should not be used in waterfowl.
(ii) *The agar gel immunodiffusion (AGID) test.* (A) The AGID test must be conducted on all ELISA-positive samples.
(B) The AGID test must be conducted using reagents approved by the Department and the Official State Agency.
(C) Standard test procedures for the AGID test for avian influenza are set forth in §147.9 of this subchapter. The test can be conducted on egg yolk or blood samples.
(D) Positive tests for the AGID must be further tested by Federal Reference Laboratories using appropriate tests for confirmation. Final judgment may be based upon further sampling and appropriate tests for confirmation.

§146.13(b)(1) ***Avian influenza*.** The official tests for avian influenza are described in paragraphs (b)(1) and (b)(2) of this section:
(1) *Antibody detection tests*—(i) *Enzyme-linked immunosorbent assay (ELISA)*. ELISA must be conducted using test kits approved by the Department and the Official State Agency and must be conducted in accordance with the recommendations of the producer or manufacturer. The AGID test should not be used in waterfowl.
(ii) *The agar gel immunodiffusion (AGID) test.*
(A) The AGID test must be conducted on all ELISA-positive samples.
(B) The AGID test must be conducted using reagents approved by the Department and the Official State Agency.
(C) Standard test procedures for the AGID test for avian influenza are set forth in §147.9 of this subchapter. The test can be conducted on egg yolk or blood samples.
(D) Positive tests for the AGID must be further tested by Federal Reference Laboratories using appropriate tests for confirmation. Final judgment may be based upon further sampling and appropriate tests for confirmation.

Reason: The AGID and ELISA tests for Avian Influenza are not reliable tests in waterfowl.

Sponsor: Senior Coordinator

Proposal No. 5

Delegates

Combined

§ 56.5 Destruction and disposal of poultry and cleaning and disinfection of premises, conveyances, and materials.

(a) Destruction of poultry. Poultry that are infected with or exposed to H5/H7 LPAI may be required to be destroyed at the discretion of the Cooperating State Agency and APHIS and in accordance with the initial State response and containment plan described in §56.10. The Cooperating State Agency and APHIS will select a method to use for the destruction of such poultry based on the following factors:

(1) The species, size, and number of the poultry to be destroyed;
(2) The environment in which the poultry are maintained;
(3) The risk to human health or safety of the method used;
(4) Whether the method requires specialized equipment or training;
(5) The risk that the method poses of spreading the H5/H7 LPAI virus;
(6) Any hazard the method could pose to the environment;
(7) The degree of bird control and restraint required to administer the destruction method;
(8) The speed with which destruction must be conducted; and
(9) Consistency of the method with humane euthanasia guidelines.

(b) Disposal of poultry. Carcasses of poultry that have died from H5/H7 LPAI infection or poultry that have been humanely slaughtered to fulfill depopulation requirements must be disposed of promptly and efficiently in accordance with the initial State response and containment plan described in §56.10 to prevent the spread of H5/H7 LPAI infection. Disposal methods will be selected by the Cooperating State Agency and APHIS and may include one or more of the following: Burial, incineration, composting, or rendering. Regardless of the method used, strict biosecurity procedures must be implemented and enforced for all personnel and vehicular movement into and out of the area in accordance with the initial State response and containment plan to prevent dissemination of the H5/H7 LPAI virus.

(c) Controlled marketing. (1) At the discretion of the Cooperating State Agency and APHIS, poultry that has been infected with or exposed to H5/H7 LPAI may be allowed to move for controlled marketing in accordance with the initial State response and containment plan described in §56.10 and in accordance with the following requirements:

(i) Poultry infected with or exposed to H5/H7 LPAI must not be transported to a market for controlled marketing until ~~21 days after the acute phase of~~ the infection or evidence of the infection has concluded, as determined by the Cooperating State Agency in accordance with the initial State response and containment plan described in §56.10; and

(ii) Within 7 days prior to slaughter, each flock to be moved for controlled marketing must be tested for H5/H7 LPAI using a test approved by the Cooperating State Agency and found to be free of the virus.

(2) Poultry moved for controlled marketing will not be eligible for indemnity under §56.3. However, any costs related to cleaning and disinfection of premises, conveyances, and materials that came into contact with poultry that are moved for controlled marketing will be eligible for indemnity under §56.3.

Reason: It is difficult to determine the acute phase of H5/H7 LPAI infections in the absence of clinical signs. The 21 day requirement has caused serious delays in the controlled marketing of infected and/or exposed flocks. This proposed change will provide the Cooperating State Agency flexibility with the controlled marketing of meat-type chicken and meat-type turkey flocks.

Proponent: Dr. Dale Lauer and Dr. Shauna Voss
Minnesota Board of Animal Health

Proposal No. 6

Delegates:

B,C,E,G,H,I **§145.23(b), 145.33(b), 145.53(b), 145.93(b)** ***U.S. Pullorum-Typhoid Clean.*** A flock in which freedom from pullorum and typhoid has been demonstrated to the Official State Agency under the criteria in one of the following paragraphs (b)(1) through (5) of this section (See §145.14 relating to the official blood test where applicable.):

(1) It has been officially blood tested within the past 12 months with no reactors.

(2) It is a multiplier breeding flock, or a breeding flock composed of progeny of a primary breeding flock which is intended solely for the production of multiplier breeding flocks, and meets the following specifications as determined by the Official State Agency and the Service:

(i) The flock is located in a State where all persons performing poultry disease diagnostic services within the State are required to report to the Official State Agency within 48 hours the source of all poultry specimens from which S. pullorum or S. gallinarum is isolated;

(ii) The flock is composed entirely of birds that originated from U.S. Pullorum-Typhoid Clean breeding flocks or from flocks that met equivalent requirements under official supervision; and

(iii) The flock is located on a premises where a flock not classified as U.S. Pullorum-Typhoid Clean was located the previous year; *Provided,* That an Authorized Testing Agent must blood test up to 300 birds per flock, as described in §145.14, if the Official State Agency determines that the flock has been exposed to pullorum-typhoid. In making determinations of exposure and setting the number of birds to be blood tested, the Official State Agency shall evaluate the results of any blood tests, described in §145.14(a)(1), that were performed on an unclassified flock located on the premises during the previous year; the origins of the unclassified flock; and the probability of contacts between the flock for which qualification is being sought and (a) infected wild birds, (b) contaminated feed or waste, or (c) birds, equipment, supplies, or personnel from flocks infected with pullorum-typhoid.

(3) It is a multiplier breeding flock that originated from U.S. Pullorum-Typhoid Clean breeding flocks or from flocks that met equivalent requirements under official supervision, and is located in a State in which it has been determined by the Service that:

(i) All hatcheries within the State are qualified as "National Plan Hatcheries" or have met equivalent requirements for pullorum-typhoid control under official supervision;

(ii) All hatchery supply flocks within the State, are qualified as U.S. Pullorum-Typhoid Clean or have met equivalent requirements for pullorum-typhoid control under official supervision: *Provided,* That if other domesticated fowl, except waterfowl, are maintained on the same premises as the participating flock, freedom from pullorum-typhoid infection shall be demonstrated by an official blood test of each of these fowl;

(iii) All shipments of products other than U.S. Pullorum-Typhoid Clean, or equivalent, into the State are prohibited;

(iv) All persons performing poultry disease diagnostic services within the State are required to report to the Official State Agency within 48 hours the source of all poultry specimens from which S. pullorum or S. gallinarum is isolated;

(v) All reports of any disease outbreak involving a disease covered under the Plan are promptly followed by an investigation by the Official State Agency to determine the origin of the infection; *Provided,* That if the origin of the infection involves another State, or if there is exposure to poultry in another State from the infected flock, then the National Poultry Improvement Plan will conduct an investigation;

(vi) All flocks found to be infected with pullorum or typhoid are quarantined until marketed or destroyed under the supervision of the Official State Agency, or until subsequently blood tested, following the procedure for reacting flocks as contained in §145.14(a)(5), and all birds fail to demonstrate pullorum or typhoid infection;

(vii) All poultry, including exhibition, exotic, and game birds, but excluding waterfowl, going to public exhibition shall come from U.S. Pullorum-typhoid Clean or equivalent flocks, or have had a negative pullorum typhoid test within 90 days going to public exhibition; except in Pullorum-Typhoid Clean States where poultry may be exempt from the requirement for Pullorum-Typhoid testing prior to exhibition.

(viii) (Discontinuation of any of the conditions or procedures described in paragraphs (b)(3)(i), (ii), (iii), (iv), (v), (vi), and (vii) of this section, or the occurrence of repeated outbreaks of pullorum or typhoid in poultry breeding flocks within or originating within the State shall be grounds for the Service to revoke its determination that such conditions and procedures have been met or complied with. Such action shall not be taken until a thorough investigation has been made by the Service and the Official State Agency has been given an opportunity to present its views.

(4) It is a multiplier breeding flock located in a State which has been determined by the Service to be in compliance with the provisions of paragraph (b)(3) of this section, and in which pullorum disease or fowl typhoid is not known to exist nor to have existed in hatchery supply flocks within the State during the preceding 24 months.

(5) It is a primary breeding flock located in a State determined to be in compliance with the provisions of paragraph (b)(4) of this section, and in which a sample of 300 birds from flocks of more than 300, and each bird in flocks of 300 or less, has been officially tested for pullorum-typhoid within the past 12 months with no reactors: *Provided,* That a bacteriological examination monitoring program or serological examination monitoring program for game birds acceptable to the Official State Agency and approved by the Service may be used in lieu of annual blood testing: *And Provided further,* That when a flock is a hobbyist or exhibition waterfowl or exhibition poultry primary breeding flock located in a State which has been deemed to be a U.S. Pullorum-Typhoid Clean State for the past three years, and during which time no isolation of pullorum or typhoid has been made that can be traced to a source in that State, a bacteriological examination monitoring program or a serological examination monitoring program acceptable to the Official State Agency and approved by the Service may be used in lieu of annual blood testing.

§ 145.73(b) and 145.83(b) *U.S. Pullorum-Typhoid Clean.*

A flock in which freedom from pullorum and typhoid has been demonstrated to the Official State Agency under the criteria in paragraph (b)(1) or (b)(2) of this section: *Provided,* That a flock qualifying by means of a blood test shall be tested within the past 12 months, except that the retesting of a participating flock which is retained for more than 12 months shall be conducted a minimum of 4 weeks after the induction of molt. (See §145.14 relating to the official blood test where applicable.)

(1) It has been officially blood tested with no reactors.

(2) It is a primary breeding flock that meets the following criteria:

(i) The primary breeding flock is located in a State in which pullorum disease or fowl typhoid is not known to exist nor to have existed in hatchery supply flocks during the preceding 12 months and in which it has been determined by the Service that:

(A) All hatcheries within the State are qualified as "National Plan Hatcheries" or have met equivalent requirements for pullorum-typhoid control under official supervision;

(B) All hatchery supply flocks within the State are qualified as U.S. Pullorum-Typhoid Clean or have met equivalent requirements for pullorum-typhoid control under official supervision: *Provided,* That if other domesticated fowl, except waterfowl, are maintained on the same premises as the participating flock, freedom from pullorum-typhoid infection shall be demonstrated by an official blood test of each of these fowl;

(C) All shipments of products other than U.S. Pullorum-Typhoid Clean, or equivalent, into the State are prohibited;

(D) All persons performing poultry disease diagnostic services within the State are required to report to the Official State Agency within 48 hours the source of all poultry specimens from which *S. pullorum* or *S. gallinarum* is isolated;

(E) All reports of any disease outbreak involving a disease covered under the Plan are promptly followed by an investigation by the Official State Agency to determine the origin of the infection; *Provided,* That if the origin of the infection involves another State, or if there is exposure to poultry in another State from the infected flock, then officials administering the National Poultry Improvement Plan will conduct an investigation;

(F) All flocks found to be infected with pullorum or typhoid are quarantined until marketed or destroyed under the supervision of the Official State Agency, or until subsequently blood tested following the procedure for reacting flocks as contained in §145.14(a)(5), and all birds fail to demonstrate pullorum or typhoid infection;

~~(~~G) All poultry, including exhibition, exotic, and game birds, but excluding waterfowl, going to public exhibition shall come from U.S. Pullorum-Typhoid Clean or equivalent flocks, or have had a negative pullorum-typhoid test within 90 days of going to public exhibition <u>except in Pullorum-Typhoid Clean States where poultry may be exempt from the requirement for Pullorum-Typhoid testing prior to exhibition.</u>

(H) Discontinuation of any of the conditions or procedures described in paragraphs (b)(2)(i)(A) through (b)(2)(i)(G) of this section, or the occurrence of repeated outbreaks of pullorum or typhoid in poultry breeding flocks within or originating within the State shall be grounds for the Service to revoke its determination that such conditions and procedures have been met or complied with. Such action shall not be taken until a thorough investigation has been made by the Service and the Official State Agency has been given an opportunity to present its views; and

Reason: If a state has been free of S. Pullorum-Typhoid for "X" years (Illinois since 1976), the flock owners in that state should see some benefit for having to test their flocks for exhibition all those years. In IL we think that benefit should be for Illinois flocks, whether in the NPIP program or not, to NOT have to test their flocks for S. Pullorum-Typhoid for exhibition at county/state fairs and shows. IL flocks exhibiting at other state fairs and shows should still have to test their flocks in compliance with that state's rules. Out – of – State flocks coming to IL for exhibition would still have to be either in the NPIP program or have their birds tested within 30 days of exhibition.

Sponsor: Dr. Robert J. Waters
Illinois Department of Agriculture

Proposal No. 7

Delegates

6C

§ 146.33 Terminology and classification; meat-type chicken slaughter plants.

(a*) U.S. H5/H7 Avian Influenza Monitored.*

This program is intended to be the basis from which the meat-type chicken industry may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of the H5/H7 subtypes of avian influenza in meat-type chickens through routine surveillance of each participating meat-type chicken slaughter plant. A meat-type chicken slaughter plant will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a meat-type chicken slaughter plant where a minimum of 11 birds per shift are tested negative for antibodies to the H5/H7 subtypes of avian influenza, as provided in §146.13(b), at slaughter; Provided, that with the approval of the Official State Agency, fewer than 11 birds per shift may be tested on any given shift if the total number of birds tested during the operating month is equivalent to testing 11 birds per shift; or

(2) It is a meat-type chicken slaughter plant which accepts only meat-type chickens from flocks where a minimum of 11 samples have been collected no more than 21 days prior to slaughter and ~~birds have been~~ tested negative for antibodies to the H5/H7 subtypes of avian influenza, as provided in §146.13(b), ~~no more than 21 days prior to slaughter;~~ or

(3) It is a meat-type chicken slaughter plant that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(1) or (a)(2) and that is approved by the Official State Agency and the Service.

Reason: There has been some misunderstanding regarding collection and test dates for the plant inspection requirements as listed in 146.11. This proposal will provide additional details to the meat-type chicken slaughter plant participation standards.

Proponent: Dr. Dale Lauer and Dr.Shauna Voss
Minnesota Board of Animal Health

Proposal No. 8

Delegates

Combined **§ 146.11 Inspections.**

(a) Each participating slaughter plant shall be audited at least once annually or a sufficient number of times each year to satisfy the Official State Agency that the participating slaughter plant is in compliance with the provisions of this part. The yearly audit will consist of an evaluation of 2 weeks' worth of records, selected at random, of the following data:

(1) The actual flock slaughter date for each flock. This information must come from a verifiable source. Verifiable sources include electronic record systems that have oversight from the Department's Grain Inspectors, Packers and Stockyards Administration or Food Safety and Inspection Service (FSIS) documents such as FSIS Form 9061–2.

(2) Laboratory test results for each flock slaughtered with the sample collection date and test result. The test must be NPIP-approved and performed in an authorized laboratory of the NPIP.

(b) A flock will be considered to be not conforming to protocol if there are no test results available, if samples from the flock were not collected within 21 days before slaughter and ~~the flock was not~~ tested ~~within 21 days before slaughter~~, or if the test results for the flocks were not returned before slaughter.

(c) Two or more flocks that are found to be not conforming to protocol in the yearly audit for a slaughter plant shall be cause for a deficiency rating for that plant. However, if the root cause for the deficiency was identified, corrected, and documented, the plant will be eligible for an immediate reevaluation of 2 additional weeks' worth of records, again selected at random. If no more than one missed flock is identified in this reevaluation, the plant will be considered in compliance and no further action will be required. Plants found to be deficient must provide a written corrective action plan to the auditor within 2 weeks of receipt of the deficiency rating. A followup audit on the information in paragraphs (a)(1) and (a)(2) of this section will occur within 90 days from the receipt of the corrective action plan. Slaughter plants will retain their classification and may continue to use the Plan emblem in §146.9(a) during this process. A failure on the followup audit may result in disbarment from participation according to the procedures in §146.12.

(d) On-site inspections of any participating flocks and premises will be conducted if a State Inspector determines that a breach of testing has occurred for the Plan programs for which the flocks are certified.

(e) The official H5/H7 LPAI testing records of all participating flocks and slaughter plants shall be examined annually by a State Inspector. Official H5/H7 LPAI testing records shall be maintained for 3 years.

Reason: There has been some misunderstanding regarding collection and test dates for the plant inspection requirements as listed in 146.11. This proposal will provide additional details to the meat-type chicken and meat-type turkey slaughter plant participation standards.

Proponent: Dr. Dale Lauer and Dr. Shauna Voss
Minnesota Board of Animal Health

Proposal No. 9

Delegates

6D **§ 146.43 Terminology and classification; meat-type turkey slaughter plants. (a) U.S. H5/H7 Avian Influenza Monitored**.

This program is intended to be the basis from which the meat-type turkey industry may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of avian influenza in meat-type turkeys through routine surveillance of each participating meat-type turkey slaughter plant. A participating meat-type turkey slaughter plant will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a meat-type turkey slaughter plant that accepts only meat-type turkeys from flocks where a minimum of 6 samples ~~6 birds~~ per flock ~~has~~ have been collected no more than 21 days prior to slaughter and tested negative for ~~antibodies to~~ type A avian influenza, as provided in §146.13(b), with an approved test ~~no more than 21 days prior to slaughter~~. Positive samples shall be further tested by ~~an~~ a Federal Reference ~~authorized~~ laboratory ~~using the hemagglutination inhibition test~~ to ~~detect antibodies to~~ determine the hemagglutinin subtypes H5 and H7. It is recommended that samples be collected from flocks over 10 weeks of age with respiratory signs such as coughing, sneezing, snicking, sinusitis, or rales; depression; or decreases in food or water intake.

(2) It is a meat-type turkey slaughter plant that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(1) and that is approved by the Official State Agency and the Service.

Reason: There has been some misunderstanding regarding collection and test dates for the plant inspection requirements as listed in 146.11. This proposal will provide additional details to the meat-type turkey slaughter plant participation standards.

Proponent: Dr. Dale Lauer and Dr. Shauna Voss
Minnesota Board of Animal Health

Delegates

D

145.43 Terminology and classification; flocks and products

(f) U.S. Sanitation Monitored, Turkeys.

A flock or hatchery whose owner is controlling or reducing the level of salmonella through compliance with sanitation and management practices as described in subpart C of part 147 of this chapter, and where the following monitoring, testing, and management practices are conducted:

(1) Hatchery debris (dead germ hatching eggs, fluff, and meconium collected by sexors), swabs collected from hatch debris in hatcher trays, a sample of all the poults that died within 10 days after hatching up to 10 poults, or a combination of 2 or all 3 of the above, from each hatch or a candidate breeding flock produced by a primary breeder, are examined bacteriologically at an authorized laboratory for Salmonella as described in §147.12 of this subchapter. All Salmonella isolates shall be serotyped.

(2) The poults for the candidate breeding flock are placed in a building that has been cleaned and disinfected. An Authorized Agent must collect environmental samples from the building and submit them to an authorized laboratory for a bacteriological examination for the presence of Salmonella, as described in §147.12 of this subchapter.

(3) Feed for turkeys in the candidate and breeding flock should meet the following requirements:

(i) All feed manufactured in pellet form must have a maximum moisture content of 13.5 percent upon delivery to the farm. It should have been preconditioned to the minimum of one of the following parameters before pelleting:

(A) Feed is to reach a minimum temperature of 185 °F for a minimum of 6 minutes of retention in the conditioning chamber. The conditioned mash feed moisture must be a minimum of 16 percent during the conditioning process. This method utilizes time retention to allow permeation to the center core of each feed particle; or

(B) The feed is to be pressurized in order to expedite the transfer of the heat and moisture to the core of each feed particle. The feed should be conditioned to the parameters of a minimum of 16 percent moisture and 200 °F; or

(C) The feed should be submitted to pressurization to the extent that the initial feed temperature rises to 235 °F for 4 seconds; or

(D) The feed should be submitted to an equivalent thermal lethality treatment; or

(E) A Food and Drug Administration (FDA)-approved product for Salmonella control should be added to the finished pellets.

(ii) Mash feed should be treated with an FDA-approved Salmonella control product.

(iii) All feed is to be stored and transported in such a manner as to prevent possible contamination with pathogenic bacteria.

(iv) FDA-approved products for Salmonella control may be added to either unfinished or finished feed.

(4) Environmental samples shall be taken by an Authorized Agent, as described in §147.12 of this chapter, from each flock at 12–20 weeks of age and every 30 days thereafter to be examined bacteriologically at an authorized laboratory for Salmonella. All Salmonella isolates shall be serotyped.

(5) Owners of flocks found infected with a paratyphoid Salmonella may vaccinate these flocks with an autogenous bacterin with a potentiating agent.

~~(6) Environmental samples shall be taken by an Authorized Agent, as described in §147.12 of this chapter, from each flock at 35–50 weeks of age and from each~~

~~molted flock at midlay, and examined bacteriologically at an authorized laboratory for Salmonella.~~

(6~~7~~) Hatchery debris (dead germ hatching eggs, fluff, and meconium collected by sexors), swabs collected from hatch debris in hatcher trays, or a sample of all the poults that died within 10 days after hatching up to 10 poults~~, or a combination of 2 or all 3 of the above,~~ shall be cultured from each hatch and examined bacteriologically at an authorized laboratory for Salmonella as a means of evaluating the effectiveness of the control procedures. All Salmonella isolates shall be serogrouped. A representative sample of Salmonella isolates will be serotyped every week.

(7) This classification may be revoked by the Official State Agency if the participant fails to comply with the all the testing requirements of this classification.

Reason: To define Salmonella testing and sanitation standards for turkey breeding flocks and hatcheries.

Proponent: Dr. Dale Lauer and Dr. Shauna Voss
Minnesota Board of Animal Health

Proposal No. 11

Delegates:

B,G **§ 145.23 Terminology and classification; flocks and products.**

(h) ***U.S. Avian Influenza Clean.***

This program is intended to be the basis from which the breeding-hatchery industry may conduct a program for the prevention and control of avian influenza. It is intended to determine the presence of avian influenza in breeding chickens through routine surveillance of each participating breeding flock. A flock and the hatching eggs and chicks produced from it will qualify for this classification when the Official State Agency determines that they have met the following requirements:

(1) It is a multiplier breeding flock in which a minimum of 30 birds have been tested and found negative ~~for antibodies~~ to avian influenza when more than 4 months of age. To retain this classification:

(i) A sample of at least 30 birds must be tested negative at intervals of 90 days; or

(ii) A sample of fewer than 30 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 30 birds is tested within each 90-day period; or

(iii) The flock is tested as provided in §145.14(d) at intervals of 30 days or less and found to be negative, and a total of 30 samples are collected and tested within each 90-day period; and

(2) A sample of at least 30 birds ~~During each 90 day period, all multiplier spent fowl, up to a maximum of 30,~~ must be tested and found negative to Avian Influenza within 21 days prior to movement to slaughter.

Reason: Approved tests for AI include antibody and antigen tests. To state requirements for slaughter testing more clearly.

Sponsor: Senior Coordinator

Proposal No. 12

Delegates:

C, H

§ 145.33 Terminology and classification; flocks and products.

(l) ***U.S. Avian Influenza Clean.***

This program is intended to be the basis from which the breeding-hatchery industry may conduct a program for the prevention and control of avian influenza. It is intended to determine the presence of avian influenza in primary breeding chickens through routine surveillance of each participating breeding flock. A flock and the hatching eggs and chicks produced from it will qualify for this classification when the Official State Agency determines that they have met the following requirements:

(1) It is a multiplier breeding flock in which a minimum of 30 birds have been tested and found negative ~~for antibodies~~ to avian influenza when more than 4 months of age. To retain this classification:

(i) A sample of at least 15 birds must be tested negative at intervals of 90 days; or

(ii) A sample of fewer than 15 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 15 ~~30~~ birds is tested within each 90-day period; or

(iii) The flock is tested as provided in §145.14(d) at intervals of 30 days or less and found to be negative, and a total of 15 samples are collected and tested within each 90-day period; and

(2) A sample of at least 30 birds ~~During each 90-day period, all multiplier spent fowel, up to a maximum of 30,~~ must be tested and found negative to Avian Influenza within 21 days prior to movement to slaughter.

Reason: Approved tests for AI include antibody and antigen tests. To state requirements for slaughter testing more clearly.

Sponsor: Senior Coordinator

Proposal No. 13

Delegates:

E, I

§ 145.53 Terminology and classification; flocks and products.

(e) *U.S. H5/H7 Avian Influenza Clean.*

This program is intended to be the basis from which the breeding-hatchery industry may conduct a program for the prevention and control of the H5 and H7 subtypes of avian influenza. It is intended to determine the presence of the H5 and H7 subtypes of avian influenza in hobbyist or exhibition waterfowl, exhibition poultry, and game bird breeding flocks through routine surveillance of each participating breeding flock. A flock, and the hatching eggs and chicks produced from it, will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a primary breeding flock in which a minimum of 30 birds has been tested negative to the H5 and H7 subtypes of avian influenza as provided in §145.14(d) when more than 4 months of age. To retain this classification:

(i) A sample of at least 30 birds must be tested negative at intervals of 90 days; or

(ii) A sample of fewer than 30 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 30 birds are tested within each 90-day period.

(2) It is a multiplier breeding flock in which a minimum of 30 birds has been tested negative to the H5 and H7 subtypes of avian influenza as provided in §145.14(d) when more than 4 months of age. To retain this classification:

(i) A sample of at least 30 birds must be tested negative at intervals of 180 days; or

(ii) A sample of fewer than 30 birds may be tested, and found to be negative, at any one time if all pens are equally represented and a total of 30 birds are tested within each 180-day period.

(3) A sample of at least 30 birds ~~During each 90 day period, all spent fowl, up to a maximum of 30,~~ must be tested and found negative to H5/H7 Avian Influenza within 21 days prior to movement to slaughter.

Reason: To state requirements for slaughter testing more clearly.

Sponsor: Senior Coordinator

Proposal No. 14

Delegates:

6B

§ 146.23 Terminology and classification; flocks and products.

(a) ***U.S. H5/H7 Avian Influenza Monitored.***

This program is intended to be the basis from which the table-egg layer industry may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of the H5/H7 subtypes of avian influenza in table-egg layers and table-egg layer pullets through routine surveillance of each participating commercial table-egg layer and table-egg layer pullet flock. A flock will qualify for this classification when the Official State Agency determines that it has met ~~one of~~ the following requirements:

(1) *Table-egg layer pullet flocks.* (i) It is a commercial table-egg layer pullet flock in which a minimum of 11 birds have been tested negative to the H5/H7 subtypes of avian influenza as provided in §146.13(b) within 30 days prior to movement; or

(ii) It is a commercial table-egg layer pullet flock that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(1)(i) of this section and that is approved by the Official State Agency and the Service.

(2) *Table-egg layer flocks.* (i) It is a commercial table-egg layer flock in which a minimum of 11 birds have been tested negative to the H5/H7 subtypes of avian influenza as provided in §146.13(b) within 30 days prior to disposal;

(ii) It is a commercial table-egg layer flock in which a minimum of 11 birds have been tested negative for the H5/H7 subtypes of avian influenza as provided in §146.13(b) within a 12-month period; or

(iii) It is a commercial table-egg layer flock that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(2)(i) or paragraph (a)(2)(ii) of this section and that is approved by the Official State Agency and the Service.

Reason: To clarify that both pullets and layers must be tested to satisfy the program.

Sponsor: Senior Coordinator

Proposal No. 15

Delegates:

6C **§ 146.33 Terminology and classification; meat-type chicken slaughter plants.**

(a) ***U.S. H5/H7 Avian Influenza Monitored.***

This program is intended to be the basis from which the meat-type chicken industry may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of the H5/H7 subtypes of avian influenza in meat-type chickens through routine surveillance of each participating meat-type chicken slaughter plant. A meat-type chicken slaughter plant will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a meat-type chicken slaughter plant where a minimum of 11 birds per shift are tested negative ~~for antibodies~~ to the H5/H7 subtypes of avian influenza, as provided in §146.13(b), at slaughter; *Provided*, that with the approval of the Official State Agency, fewer than 11 birds per shift may be tested on any given shift if the total number of birds tested during the operating month is equivalent to testing 11 birds per shift; or

(2) It is a meat-type chicken slaughter plant which accepts only meat-type chickens from flocks where a minimum of 11 birds have been tested negative ~~for antibodies~~ to the H5/H7 subtypes of avian influenza, as provided in §146.13(b), no more than 21 days prior to slaughter; or

(3) It is a meat-type chicken slaughter plant that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(1) or (a)(2) and that is approved by the Official State Agency and the Service.

Reason: Official test include antibody and antigen tests.

Sponsor: Senior Coordinator

Proposal No. 16

Delegates:

6D

§ 146.43 Terminology and classification; meat-type turkey slaughter plants.

(a) ***U.S. H5/H7 Avian Influenza Monitored.***

This program is intended to be the basis from which the meat-type turkey industry may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of avian influenza in meat-type turkeys through routine surveillance of each participating meat-type turkey slaughter plant. A participating meat-type turkey slaughter plant will qualify for this classification when the Official State Agency determines that it has met one of the following requirements:

(1) It is a meat-type turkey slaughter plant that accepts only meat-type turkeys from flocks where a minimum of 6 birds per flock has tested negative ~~for antibodies~~ to the H5/H7 subtypes of ~~A~~ avian influenza, as provided in §146.13(b), with an approved test no more than 21 days prior to slaughter. Positive samples shall be further tested by an authorized laboratory using the hemagglutination inhibition test to detect antibodies to the hemagglutinin subtypes H5 and H7. It is recommended that samples be collected from flocks over 10 weeks of age with respiratory signs such as coughing, sneezing, snicking, sinusitis, or rales; depression; or decreases in food or water intake.

(2) It is a meat-type turkey slaughter plant that has an ongoing active and diagnostic surveillance program for the H5/H7 subtypes of avian influenza in which the number of birds tested is equivalent to the number required in paragraph (a)(1) and that is approved by the Official State Agency and the Service.

Reason: Official test include antibody and antigen tests.

Sponsor: Senior Coordinator

Proposal No. 17

Delegates:
6E

§ 146.51 Definitions.

Exhibition Poultry. Domesticated fowl which are bred for the combined purposes of meat or egg production and competitive showing

§ 146.52 Participation. (a) Participating commercial upland game bird slaughter plants, commercial waterfowl slaughter plants, raised-for-release upland game bird premises, and raised-for-release waterfowl premises shall comply with the applicable general provisions of Subpart A of this part and the special provisions of this subpart E.
(b) Commercial waterfowl and commercial upland game bird slaughter plants that slaughter fewer than 50,000 birds annually are exempt from the special provisions of this subpart E.
(c) Raised-for-release upland game bird premises and raised-for-release waterfowl premises that raise fewer than 25,000 birds annually are exempt from the special provisions of this subpart E.
(d) Exhibition poultry that are not breeding birds and meets the definition in §146.51.

§ 146.53 Terminology and classification; slaughter plants and premises.
(b) *U.S. H5/H7 Avian Influenza Monitored*.
This program is intended to be the basis from which the exhibition poultry industry;the raised-for-release upland game bird and the raised-for-release waterfowl industries may conduct a program to monitor for the H5/H7 subtypes of avian influenza. It is intended to determine the presence of the H5/H7 subtypes of avian influenza through routine surveillance of each participating premises. A premises will qualify for the classification when the Official State Agency determines that a representative sample of 30 birds from the participating premises has been tested with negative results for the H5/H7 subtypes of avian influenza, as provided in §146.13(b), every 90 days.

Reason: To include exhibition, show and competition birds in the H5/H7 program.

Sponsor: Kim Arnold
Marayland Department of Agriculture

Proposal No. 18

Delegates:
Combined **§56.1, 145.1 and 146.1 Definitions**

146.1

H5/H7 low pathogenic avian influenza (LPAI). An infection of poultry caused by an influenza A virus of H5 or H7 subtype that has an intravenous pathogenicity index test in 6-week-old chickens less than 1.2 or less than 75 percent mortality in 4 to 8 week old chickens infected intravenously, or an infection with influenza A viruses of H5 or H7 subtype with a cleavage site that is not consistent with a previously identified highly pathogenic avian influenza virus.

56.1

H5/H7 low pathogenic avian influenza (LPAI). An infection of poultry caused by an influenza A virus of H5 or H7 subtype that has an intravenous pathogenicity index test in 6-week-old chickens less than 1.2 or less than 75 percent mortality in 4 to 8 week old chickens infected intravenously, or any infection with influenza A viruses of H5 or H7 subtype with a cleavage site that is not consistent with a previously identified highly pathogenic avian influenza virus. ~~for which nucleotide sequencing has not demonstrated the presence of multiple basic amino acids at the cleavage site of the hemagglutinin.~~

145.1

H5/H7 low pathogenic avian influenza (LPAI). An infection of poultry caused by an influenza A virus of H5 or H7 subtype that has an intravenous pathogenicity index test in 6-week-old chickens less than 1.2 or less than 75 percent mortality in 4 to 8 week old chickens infected intravenously, or an infection with influenza A viruses of H5 or H7 subtype with a cleavage site that is not consistent with a previously identified highly pathogenic avian influenza virus.

Reason: The proposed definition is the current definition from Part 146, which is the most recently amended definition from the 2008 Conference. Amending the definition will establish a consistent definition for LPAI in Parts 145, 146 and 56.

Sponsor: Jennifer Hall
Kentucky Poultry Association

Proposal No. 19

Delegates:

E **§ 145.52 Participation.**

Participating flocks of hobbyist and exhibition waterfowl, exhibition poultry, and game birds, and the eggs and baby poultry produced from them shall comply with the applicable general provisions of subpart A of this part and the special provisions of this subpart E. The special provisions that apply to meat-type waterfowl flocks are found in subpart I of this part.

(a) Started poultry shall lose their identity under Plan terminology when not maintained by Plan participants under the conditions prescribed in §145.5(a).

(b) Hatching eggs produced by primary breeding flocks shall be fumigated (see §147.25 of this chapter) or otherwise sanitized.

(c)Waterfowl flocks and gallinaceous flocks housed in open air facilities should be kept separate.

(~~c~~d) Subject to the approval of the Service and the Official State Agencies in the importing and exporting States, participating flocks may report poultry sales to importing States by using either VS Form 9–3, "Report of Sales of Hatching Eggs, Chicks, and Poults," or by using a hatchery invoice form (9–3I) approved by the Official State Agency and the Service to identify poultry sales to clients. If the selling hatchery uses the 9–3I form, the following information must be included on the form:

(1) The form number "9–3I", printed or stamped on the invoice;

(2) The hatchery name and address;

(3) The date of shipment;

(4) The hatchery invoice number;

(5) The purchaser name and address;

(6) The quantity of products sold;

(7) Identification of the products by bird variety or by NPIP stock code as listed in the NPIP APHIS 91–55–078 appendix; and

(8) The appropriate NPIP illustrative design in §145.10. One of the designs in §145.10(b) or (g) must be used. The following information must be provided in or near the NPIP design:

(i) The NPIP State number and NPIP hatchery approval number; and

(ii) The NPIP classification for which product is qualified (e.g., U.S. Pullorum-Typhoid Clean).

(~~d~~e) Any nutritive material provided to baby poultry must be free of the avian pathogens that are officially represented in the Plan disease classifications listed in §145.10.

Reason: Waterfowl should be kept separate from gallinaceous birds due to the danger of spread of AI virus.

Proponent: Dr. Marilyn Simunich
Idaho State Department of Agriculture

Proposal No. 20

Delegates:
Combined

§ 147.51 Authorized laboratory minimum requirements.

These minimum requirements are intended to be the basis on which an authorized laboratory of the Plan can be evaluated to ensure that official Plan assays are performed and reported as described in this part A satisfactory evaluation will result in the laboratory being recognized by the NPIP office of the Service as an authorized laboratory qualified to perform the assays provided for in this part.

(a) *Check-test proficiency*. The laboratory must use a regularly scheduled check test for each assay that it performs.

(b) *Trained technicians*. The testing procedures at the laboratory must be run or overseen by a laboratory technician who has attended and satisfactorily completed Service-approved laboratory workshops for Plan-specific diseases within the past 4 ~~3~~ years.

Reason: Both state and federal budget cuts have made it difficult to provide and attend workshops. A four year interval would not adversely affect laboratory technician performance but would make it easier for states to comply and reduce the burden on the NPIP staff.

Sponsor: Senior Coordinator

Proposal for Additions/Edits to Program Standards Document

Proposal 1

Delegates:

Combined ***(formerly) § 147.34:*** **Laboratory Procedures recommended for conventional polymerase chain reaction test for *Salmonella Enteritidis* (Now in effect by interim rule)**

Proposed Amendment:

Allows the use of a second primer set suitable for a real-time polymerase chain reaction (PCR) application that targets a region already approved for conventional PCR detection of *Salmonella Enteritidis*.

§ 147.32: **Laboratory Procedures recommended for conventional polymerase chain reaction test for *Salmonella***

(d) Primer Selection:

The SE specific primers are:
sdf I (forward) – TGTGTTTTATCTGATGCAAGAGG
sdf I (reverse) – CGTTCTTCTGGTACTTACGATGAC.
The internal control primers are:
rpl I (forward) – GGGTGATCAGGTTAACGTTAAAG
rpl I (reverse) – CTTCGTGTTCGCCAGTGGTACGC.

Or alternatively in a real time SYBR based PCR assay the following primers may be utilized F2 TTG ATG TGG TTG GTT CGT CAC T , R2 TCC CTG AAT CTG AGA AAG AAA AAC TC

(h) Alternative methods (equipment and reactions components) may be utilized by an approved laboratory as long as appropriate PCR primers as listed in 147.32 are utilized and a Group D Salmonella proficiency test provided by the service has been passed utilizing the method indicating the laboratory is performing equivalent or better detection levels with their desired PCR method. If using real time PCR assays Quantitative positive controls should be made to use with each run of this assay from a known Salmonella enteritidis Strain (as confirmed by NVSL) , or ATCC Strain extracted using Qiagen DNeasy® Tissue Kit Cat. No. 69506 or equivalent.) Records shall be maintained to show the production of the controls and consistency of the reactions of said controls in this assay over time to the Official State Agency upon request

Justification:
The primers listed in proposal 7of the 40th plan conference target the *Sdf I* DNA sequence of *Salmonella Enteritidis*, a region that reliably distinguishes *Salmonella Enteritidis* from other Salmonella subspecies. However, the referenced primers are optimized for a conventional PCR application that generates a 295bp amplicon. Real-time applications, which allow for a more rapid turn-around time, work most efficiently when amplifying target regions no larger than 100-250bp. We thus propose approving the use of primers also targeting this region but suitable for a real-time application, as published in the Journal of Applied Microbiology.

References: Yang *et al.*, J. Appl. Microbiol., 109(5): 1365-2672, 2010
Proponents:
Undine Klaube and Brenda Flack (Microbiologist Cobb-Vantress Inc., Siloam Springs AR)

Referencing *CFR Title 9 Part 147 Subpart B § 147.12 (b) Isolation and identification of Salmonella*

Proposed Modification: *(ii)* After selective enrichment, inoculate selective plates (such as BGN ~~and~~ XLT4). , and chromagenic agar. Incubate the plates at 37 °C for 20 to 24 hours. ~~Inoculate three to five *Salmonella* suspect colonies from the plates into triple sugar iron (TSI) and lysine iron agar (LIA) slants. Incubate the slants at 37 °C for 20 to 24 hours. Screen colonies by serological (*i.e.*, serogroup) and biochemical (e.g., API) procedures as shown in~~ Screen three to five colonies by serological (*i.e.*, serogroup) procedures as shown in illustration 2. A pure culture passage on a non selective or selective plate shall be performed prior to submitting any isolates to the service (NVSL) or an alternative laboratory for serotype confirmation to assure submission of pure cultures of Salmonella to the reference labs.

Justification: Triple Sugar Iron and Lysine Iron Agar slants may be replaced by another form of biochemical identification such as a chromogenic agar utilizing alternative biochemical pathways. Chromogenic agar is based on soluble colorless molecules, composed of a substrate and a chromophore. This alternative biochemical reaction uses the target organism's specific enzymes to cleave the colorless chromogenic conjugate, and a chromophore is released. In its unconjugated form, the chromophore exhibits a distinctive color; the distinctive color produced is specific to the biochemical activity of *Salmonella*. This method still provides biochemical evidence that laboratory cultures are *Salmonella* as previously provided by Triple Sugar Iron and Lysine Iron Agar slants, confirming that continuation to serotyping is appropriate.

Proponent: Jeron Freiburger (Microbiologist Cobb-Vantress Inc., Siloam Springs AR)

Proposal 2

Delegates:

Combined **(formerly) 147.32 to go in effect by interim rule if recommended by 41st NPIP technical committee and approved by GCC and plan conference delegates.**

Laboratory Procedure recommended to produce Proficiency "Ring Trial" Sample sets for establishing inter-laboratory equivalence in molecular Identification of Plan diseases sampled in the poultry upper respiratory tract.

A participant in any State wishing to work with their Official State Agency may use the following method to set up inter-laboratory proficiency sample sets to have a quantitative means to assure molecular based detection assays are detecting similar levels in laboratories providing service in a given region and be linked to current antigens provided by the Service for use in the NPIP.

a) A fresh batch of desired antigen shall be ordered from the NVSL in Ames Iowa http://www.aphis.usda.gov/animal_health/lab_info_services/downloads/AmesReagentManualCurrent.pdf (Mycoplasma gallisepticum hemagglutination (HA) Reagent code 100, Mycoplasma synoviae (HA) reagent code 120, Mycoplasma Meleagridis (HA) antigen reagent code 110, Infectious Laryngotracheitis Virus (LIT) Reagent code (033-ADV) Mycoplasma HA antigens should be thawed when received and immediately re-packaged into smaller aliquots of 250 micro liter (ul) volume and refrozen in a -70 Degree C freezer with appropriate identification. KEEP in mind when working with these HA antigens that they are live organisms and can transmit the disease + are a huge concentration of target DNA. Use appropriate lab biosecurity.

b) For each sample set to be produced one should go to a "CLEAN" turkey or chicken flock and collect 75 swabs (normal type utilized by laboratory but preference should be towards a dry packed rayon tipped swab with plastic shaft packaged in individual tubes or plastic pouches), from the birds choanal cleft region (palatine fissure), gently turning around collecting mucus/cells but ***AVOID*** getting the swabs bloody as this can interfere and cause false reactions with some assay types in the program (specifically the AI ACIA test). These will now be referred to as "CP" swabs. It is not necessary to use targeted animals for this as the objective is just to be collecting background turkey or chicken cells and accompanying respiratory region microbes.

c) Return the swabs to the lab as early in the day as possible for processing and shipment to other laboratories if desired.

d) Back in the lab, thaw one of the aliquots of HA antigen or the vial of ILT and start a 10-Fold dilution series by initially placing a volume of 100 micro-liters (ul) of the thawed well mixed antigen into 9,900 ul of PBS (147.7 (d) recipe works well for PBS). (Initial 1:100 dilution (10^{-2})) is followed after good mixing by transfer of 100ul diluted antigen into 900 ul volumes of PBS and continuing out until one reaches the 10^{-6} point.

e) Starting with a tube of 1 ml of plain PBS take three of the CP swabs with your choice of poultry already loaded onto them and add a **10 u**l volume of this PBS slowly to the tip of the swab gently turning to let it absorb into the swab tip material and return each swab back into its original pouch or tube with a temporary id as "PBS".

f) Next do the same as last step but use three swabs with the 10^{-6} dilution of desired antigen and return these to their temporary marked tubes or pouches as well. Continue with three of each swabs and dilution working towards the most concentrated ones and stop at 10^{-3}.

g) Next step is to make test "pool" sets by adding one of the PBS or Antigen loaded swabs + 4 of the "Poultry only" swabs to make a test pool set of 5 swabs to be kept in their individual packages but linked together in a small bag or with rubber bands. (Need to keep track of the PBS (negative control) or antigen concentration identification of the one swab and the code applied to the pool if doing a "ring trial set up".) (Avoid leaving your temporary identification on the individual swabs with the added PBS or antigen.)

h) Pack up all the pool samples sets and save overnight in the frig or ship via overnight mail for DNA extraction process and PCR analysis the next day.

i) It is wise to do a small test with your antigens and a minimized sample set before any big project to try and get the desired dilution range down. (May need to re-adjust the recommended above it does not hit the desired goal of finding the end point in your system and that of the other labs. This is also a great way to evaluate different swab types. It does make a difference which type one uses. Plain cotton swabs are really not desirable as you will see if you try this out.

j) Have the extraction and PCR / molecular method done as normal in the lab. (If they desire different pool sizes or not to pool at all one should know this prior to setting up the test ring set).

Reason: Currently the Service is not providing any proficiency testing sample sets to the NPIP approved Poultry diagnostic laboratories servicing our Industry to assure equivalence between laboratories in detection of plan diseases assayed by molecular diagnostic testing. This crude method which is linked to APHIS / NVSL reagents will give the Official State Agency an opportunity to do this until a more suitable method is put forth.

Submitted: Joseph Schultz, Cobb-Vantress Siloam Springs A

Proposal 3

Delegates:
Combined **(FORMERLY) CFR 147.30 –Laboratory procedure recommended for the polymerase chain reaction (PCR) test for Mycoplasma gallisepticum and M. synoviae.**

(a) DNA isolation. Isolate DNA from 1 mL of eluate from tracheal swabs in PBS or 1 mL of broth culture by a non-phenolic procedure. Centrifuge samples at 14,000 x g for 5 to 10 minutes. Decant supernatant and wash the pellet with 1 mL of PBS. Centrifuge as above and re-suspend the pellet in 25-50 µl of 0.1 percent DEP (Diethyl Pyrocarbonate; Sigma) water. Boil at 120 100 °C for 10 minutes followed by 10 minutes incubation at 4 °C. Centrifuge as above and transfer the supernatant DNA to a nuclease-free tube. Estimate the DNA concentration and purity by spectrophotometric reading at 260 nm and 280 nm.
(b) Primer selection. (1) M. gallisepticum. The primer for M. gallisepticum should consist of the following sequences:

MG-F 5' GAG CTA ATC TGT AAA GTT GGT C
MG-R 5' GCT TCC TTG CGG TTA GCA AC

(2) M. synoviae. The primer for M. synoviae should consist of the following sequences:

MS–F 5' GAG AAG CAA AAT AGT GAT ATC A
MS–R 5' CAG TCG TCT CCG AAG TTA ACA A

(c) Polymerase chain reaction. (1) Treat each sample (100 to 2000 ng/5 µl) with one of the following 45 µl PCR cocktails:
(i) 5 µl 10x PCR buffer, 1 µl dNTP (10 mM), 1 µl of Reverse primer (50 µM), 1 µl of Forward primer (50 µM), 4 µl MgCl2 (25 mM), 1 µl taq-polymerase (5 U), 32 µl DEP water.
(ii) 18 µl water, 25 µl PCR mix (Promega), 1 µl Reverse primer (50 µM), 1 µl Forward primer (50 µM).
(2) Perform DNA amplification in a Perkin-Elmer 9600 thermocycler or in a Hybaid PCR Express thermocycler. 21 The optimized PCR program is as follows:

Footnote(s): 21 Trade names are used in these procedures solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

Temperature (°C)	Duration	Cycles
94	30 seconds	30-40.
55	30 seconds	30-40.
72	1 minute	30-40.

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72	5 minutes	1 (final extension).

(d) Electrophoresis. Mix PCR products (5 to 10 µl) with 2 µl loading buffer (Sigma) and electrophorese on a 2 percent agarose gel containing 0.5 µg/mL ethidium bromide in TAE buffer (40 mM tris; 2 mM EDTA; pH 8.0 with glacial acetic acid) for 30 minutes at 80 V. M. gallisepticum (185 bp) and M. synoviae (214 bp) amplicons can be visualized under an ultraviolet transilluminator along with the PCR marker (50 to 2000 bp; Sigma).

[72 FR 1425, Jan. 12, 2007, as amended at 74 FR 14718, Apr. 1, 2009; 76 FR 15797, Mar. 22, 2011]

(e) Alternative methods (equipment and reactions components) may be utilized by an approved laboratory as long as the listed MG and MS PCR primers in 147.30 are utilized and a ring trial as outlined in 147.33 has been carried out to the satisfaction of Official State Agency and the service indicating the laboratory is performing equivalent or better detection levels with their desired PCR method. Quantitative positive controls should be made to use with each run of this assay from the Hemagglutination antigens provided from the Service. Records shall be maintained over time to show the production of the controls and consistency of the reactions of said controls in this assay over time.

Reason: While the above original method does work, (Other than the line to boil at 120 degrees C.....boiling is at 100 degrees C unless in autoclave), improvements to equipment , alternative equipment and reaction chemistries continue to evolve. This change as underlined keeps the most important component of the procedure the specific primers in place yet allows program flexibility should the Official State Agency be looking for a specific program SOP to be followed. Laboratories should also be using controls quantitatively linked to the reagents provided by the service where possible.

Submitted: Joseph Schultz, Cobb-Vantress Siloam Springs AR

Proposal 4

Delegates:
Combined **Proposed modification to the(formerly) 147.31 Procedure to reflect usage in alternative PCR reactions as stated in underlined sections.**

9 CFR 147.31 - Laboratory procedures recommended for the real-time polymerase chain reaction test for Mycoplasma gallisepticum (MGLP ReTi).

(a) DNA extraction. Use Qiagen Qiamp Mini Kit for DNA extraction or equivalent validated technique/procedure. This kit utilizes the following methods: 100 µl of swab suspension incubates with 10 µl of proteinase K and 400 µl of lysis buffer at 56 °C for 10 minutes. Following incubation, 100 µl of 100 percent ethanol is added to lysate. Wash and centrifuge following extraction kit recommendations.
(b) Primer selection. A forward primer mglpU26 (5′-CTA GAG GGT TGG ACA GTT ATG-3′) located at nucleotide positions 765,566 to 765,586 of the M. gallisepticum R strain genome sequence; a reverse primer mglp164 (5′-GCT GCA CTA AAT GAT ACG TCA AA-3′) located at nucleotide positions 765,448 to 765,470 of the M. gallisepticum R strain genome sequence; and a Taqman dual-labeled probe mglpprobe (5′-FAM-CAG TCA TTA ACA ACT TAC CAC CAG AAT CTG-BHQ1-3′) located at nucleotide positions 765,491 to 765,520 of the M. gallisepticum R strain genome should be used to amplify a 139-bp fragment of the lp gene.
(c) MGLP ReTi. Primers and probe should be utilized in a 25 µl reaction containing 12.5 µl of Quantitect Probe PCR 2X mix (Qiagen, Valencia, CA), 22 primers to a final concentration of 0.5 µmolar, and probe to a final concentration of 0.1 µmolar, 1µl of HK-UNG Thermolabile Uracil N-glycosylase (Epicentre, Madison, WI), 2 µl of water, and 5 µl of template. The reaction can be performed in a SmartCycler (Cepheid, Sunnyvale, CA) or other equivalent validated platform procedure for real-time thermocycler at 50 °C for 2 minutes; 95 °C for 15 minutes with optics OFF; and 40 cycles of 94 °C for 15 seconds followed by 60 °C for 60 seconds with optics ON.

Footnote(s): 22 Trade names are used in these procedures solely for the purpose of providing specific information. Mention of a trade name does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or an endorsement over other products not mentioned.

(d) Determination of positive. For each MGLP ReTi assay reaction, the threshold cycle number (CT value) was determined to be the PCR cycle number at which the fluorescence of the reaction exceeded 30 units of fluorescence. For all samples tested, any MGLP reaction that has a recorded CT value was considered positive, while any MGLP reaction that had no recorded CT value was considered negative.
(e) Controls. Proper controls should be used when conducting the MGLP ReTi assay as an official test of the Plan. Positive, quantitative, extraction, and internal

controls are commercially available from GTCAllison, LLC, Mocksville, NC. Controls may also be made by extraction of MG Hemaglutination antigen provided by NVSL (Reagent Code 100)
[74 FR 14718, Apr. 1, 2009, as amended at 76 FR 15797, Mar. 22, 2011]

(f) Alternative methods (equipment and reactions components) may be utilized by an approved laboratory as long as the listed MG PCR primers in 147.31 are utilized and a ring trial as outlined in 147.33 has been carried out to the satisfaction of Official State Agency and the service indicating the laboratory is performing equivalent or better detection levels with their desired PCR method.

Reason: While the above original method does work well, improvements to equipment , alternative equipment and reaction chemistries continue to evolve. The suggested change as underlined keeps the most important component of the procedure the specific primers in place yet allows program flexibility should the Official State Agency be looking for a specific program SOP to be followed.

Submitted: Joseph Schultz, Cobb-Vantress Siloam Springs AR

Proposal 5

Delegates:
Combined **addition to § 147.52 Approved tests.**

(a) The procedures for the bacteriological examination of poultry and poultry environments described in this part are approved tests for use in the NPIP. In addition, all tests that use veterinary biologics (e.g., antiserum and other products of biological origin) that are licensed or produced by the Service and used as described in this part are approved for use in the NPIP.

(b) Diagnostic test kits that are not licensed by the Service (e.g., bacteriological culturing kits) may be approved through the following procedure:

(1) The sensitivity of the kit will be estimated in at least three authorized laboratories selected by the Service by testing known positive samples, as determined by the official NPIP procedures found in Subparts A, B, C, and D of this part. If certain conditions or interfering substances are known to affect the performance of the kit, appropriate samples will be included so that the magnitude and significance of the effect(s) can be evaluated.

(2) The specificity of the kit will be estimated in at least three authorized laboratories selected by the Service by testing known negative samples, as determined by the official NPIP procedures found in this part. If certain conditions or interfering substances are known to affect the performance of the kit, appropriate samples will be included so that the magnitude and significance of the effect(s) can be evaluated.

(3) The kit will be provided to the cooperating laboratories in its final form and include the instructions for use. The cooperating laboratories must perform the assay exactly as stated in the supplied instructions. Each laboratory must test a panel of at least 25 known positive clinical samples supplied by the manufacturer of the test kit. In addition, each laboratory will be asked to test 50 known negative clinical samples obtained from several sources, to provide a representative sampling of the general population. The identity of the samples must be coded so that the cooperating laboratories are blinded to identity and classification. Each sample must be provided in duplicate or triplicate, so that error and repeatability data may be generated.

(4) Cooperating laboratories will submit to the kit manufacturer all raw data regarding the assay response. Each sample tested will be reported as positive or negative, and the official NPIP procedure used to classify the sample must be submitted in addition to the assay response value.

(5) The findings of the cooperating laboratories will be evaluated by the NPIP technical committee, and the technical committee will make a recommendation regarding whether to approve the test kit to the General Conference Committee.

If the technical committee recommends approval, the final approval will be granted in accordance with the procedures described in §§147.46 and 147.47.

(c) The following diagnostic test kits that are not licensed by the Service (e.g., bacteriological culturing kits) are approved for use in the NPIP:

(1) Rapid Chek©Select TMSalmonella Test Kit, Strategic Diagnostics, Inc., Newark, DE 19713.

(2) ADIAFOOD Rapid Pathogen Detection System for *Salmonella* spp., AES Chemunex Canada. Laval, QC (Canada) H7L4S3.

(3) DuPont Qualicon BAX Polymerase Chain Reaction (PCR)-based assay for *Salmonella,* DuPont Qualicon, Wilmington, DE 19810.

(4) Neogen Reveal 2.0 *Salmonella* Enteritidis Test, Neogen Corp., Lansing, MI 48912.

(5) Applied Biosystems TaqMan® Salmonella Enteritidis Real-Time PCR assay for the detection of Salmonella Enteritidis. Life Technologies Corporation. Foster City, CA 94404

(6) SDIX RapidChek® SelectTM Salmonella Enteritidis Test System. Strategic Diagnostics Inc. Newark DE 19702

Subject to Tech Committee Review

(1) Neogen ANSR *Salmonella* Test, Neogen Corp., Lansing, MI 48912.

(2) Idexx MG/MS RT-PCR

Proposal 6

Delegates:
Combined Subpart D- formerly 147.36 **Use of rRt-PCR for AI testing in Waterfowl**

The National Poultry Improvement Plan supports the use of cloacal swabs from domestic ducks and poultry as an approved specimen for the rRT-PCR matrix test assay when performed with the Ambion MagMAX magnetic bead procedure for the NPIP NAI US H5/H7 Avian Influenza Clean and the US H5/H7 Avian Influenza Monitored Programs. The rRT-PCR procedure will remain a screening test and all positive findings will need to be confirmed by the NVSL with rRT-PCR and virus isolation testing.

Reason:
On Nov. 3, 2011, the General Conference Committee of the National Poultry Improvement Plan reviewed research by Janice Pedersen, et. al., at the National Veterinary Services Laboratories, USDA, APHIS entitled "Comparison of Virus Isolation and Real-Time RT-PCR for Detection of Avian Influenza Virus and Newcastle Disease Virus in Cloacal Swabs of Poultry and Ducks". Based on the data collected by NVSL, the rRT-PCR test proved to be at least as sensitive as virus isolation for the detection of AI and ND viruses from domestic duck and chicken cloacal swabs when the RNA extraction is conducted with the Ambion MagMAX magnetic bead procedure. The rRT-PCR test is valid when five cloacal swabs are collected from a single species and pooled per tube.
Based on these findings the General Conference Committee of the National Poultry Improvement Plan supports the use of cloacal swabs from domestic ducks and poultry as an approved specimen for the rRT-PCR matrix test assay when performed with the Ambion MagMAX magnetic bead procedure for the NPIP NAI US H5/H7 Avian Influenza Clean and the US H5/H7 Avian Influenza Monitored Programs. The rRT-PCR procedure will remain a screening test and all positive findings will need to be confirmed by the NVSL with rRT-PCR and virus isolation testing.
The GCC is hereby giving interim approval for this testing as of Nov. 3, 2011 which will be effective until the proposal is reviewed and voted upon at the next Biennial Conference in September of 2012.

Sponsor: GCC